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Antioxidant defense of pycnogenol against glycerol induced acute renal failure in mice.

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Abstract

Introduction : The present study aims to demonstrate the efficancy of pycnogenol as a protector in male mice against hazzards effect in kidney functions induced by glycerol.

Materials And Methods: Mice were received i.m. injection 50% glycerol (8ml / Kg b.wt) 30 min. prior to glycerol administration, other group received orally pycnogenol (400mg / Kg b.wt) over a period of 12 hrs.. Lipid peroxidation products (MDA, PC), non enzymatic and enzymatic antioxidant (GSH, CAT, SOD) were estimated, in addtion serum protein, urea, createnine concentration as well as serum Na, K levels were determinated. It seems that rhabdomyolysis effect caused by glycerol can be controlled to some extent by pycnogenol administration.

Key words: Acute Renal Failure, Glycerol, Pycnogenol, Rhabdomyolysis.

Introduction

The precise mechanisms responsible for rhabdomyolysis are not fully understood, it is implicated as a major cause of ARF (Bonventre *et al.*, 1995).The most common causes of rhabdomyolysis are prodigious exercise, trauma, and alcohol abuse (Holt *et al.*, 1999). Glycerol is the backbone of triglycerols and phospholipids. These substances are present in most life forms, and dietary intake of glycerol comes mainly from these molecules in animal and plant products. The main effect of glycerol itself results from its dehydrating activity, especially oral glycerol (Robergs and Griffin , 1998). Intramuscular glycerol has a very toxic effect on muscles and kidney resulting in rhabdomyolysis and ARF (Zager, 1996). He observed that, the injection with glycerol brought about both a reduction in membrane fluidity and increase in lipid peroxidation (LPO) products in renal cells of rats. One of the key compounds released is myoglobin, a heme-containing protein that plays a major role in development of rhabdomyolysis-induced acute renal failure (ARF) (Slater and Mullins, 1998). Singh *et al.* (2004) noted that, reactive oxygen intermediates has been demonstrated to play an etiological role in myoglobinuric acute renal failure. Taysi *et al.* (2003) showed that, the process of LPO is one of oxidative conversion of poly unsaturated fatty acids to several products including Malondialdhyde (MDA), protein carbonyl (PC), and lipid peroxides. Hogg *et al.* (1994) characterized the capacity of glycerol to release of free ferrous (Fe^{++}) iron, resulting in generation of hydroxyl radicals via the Fenton reaction, or by redox cycling of ferric (Fe^{+++}) myoglobin to lipid peroxidation, inducing ferryl ($[Fe=O]^{++}$) myoglobin. Also a heme-protein induced nitric oxide (NO) scavenging may inhibits NO-induced vasodilatation resulting in Renal vasoconstriction (Gorbunov *et al.*, 1995). Molitoris *et al.* (1992) reported that, intra-muscular glycerol induced rhabdomolysis resulted in mislocalization of ionic pumps, relocation of sodium-potasium ATPase to apical membrane, and cell necrosis. Rodrigo *et al.* (2002) concluded that, a single intramuscular injection of 50% glycerol with a dose of 8 ml/kg b.wt induces oxidative cell damage in male rats, as demonstrated by an increase in Thiobarbituric acid reactive substances (TBARS) and a decrease in GSH after 6 h. of glycerol injection. Shanley (1996) suggested that, the reactive species which are reactive and damaging compounds, normal byproduct of cellular metabolic processes, are kept under control by antioxidant enzymes such as Superoxide

dismutase (SOD) and catalase (CAT). Masquelier (1979) reported that, pycnogenol is a natural product made from the bark of the European coastal pine, Pinus maritima. Packer *et al.* (1999) show that, Pycnogenol can protect against the effects of early aging, strengthen capillaries, veins, and arteries; improve circulation and skin smoothness; fight inflammation; and improve joint flexibility, binds to collegen fibers, which improves the elasticity and integrity of connective tissues , lowering cholesterol, anti-diabetic agent, reducing blood pressure, improve fertility in men, treat Alzheimer's disease and as a powerful antioxidant. Pycnogenol is rich in proanthocyanidins, a special class of water-soluble highly bioavailable antioxidant flavonoids, which are excellent free radical scavengers (Nishioka *et al.*, 2007). Since glycerol up regulates renal antioxidant enzymes and pycnogenol behave as an antioxidant, this study was designed to investigate the protective effect of pycnogenol on glycerol-induced myoglobinuric ARF in male mice and to demonstrate the relationship of oxidative stress to renal dysfunction.

Materials And Methods

Adult male mice weighing 30-35 g were divided into four groups as following: Control group, Pycnogenol (tablet) administrated group: (400 mg / kg b.wt) oral administration, Glycerol injected group: intramuscular injection of 50% glycerol (8 ml/kg b.wt) as a single dose, pycnogenol injected with glycerol administered group : Oral administration of pycnogenol (400 mg/kg b.wt) as a single dose followed by intramuscular injection of 50% glycerol (8 ml/kg body wt) as a single dose after 30 minutes from pycnogenol administration. Six hours after glycerol injection, mice were sacrificed using a sharp razor blade. Blood samples were collected in clean centrifuge tubes contaning one drop of EDTA as an anticoagulant, then the tubes were let to stand for 15 min at 30˚C after which the tubes were centrifuged at 3000 rpm for 15 min. Blood plasma were carefully separated. Aliquots of each sample were labeled and kept at - 20˚C for subsequent analysis. The following parameters were estimated :- kidney malondialdehyde (MDA); Ohkawa *et al.* (1982), kidney protein carbonyl (PC); Smith *et al.* (1991), kidney Glutathione (GSH) activity; Prins and Loose (1969), kidney Superoxide dismutase (SOD) activity; Niskikimi *et al.* (1972), kidney Catalase (CAT) activity; Bock *et al.* (1980), plasma total protein; Henry (1964), plasma electrolytes (Na & K); Tietz *et al.* (1992), plasma urea nitrogen (PUN); Henry (1974) a, plasma urea; Patton and Crouch (1977), and plasma creatinine; Henry (1974) b. The results are expressed as $\bar{X}$±SEM. The sources of variation for multiple comparisons were assessed by one-way analysis of variance (ANOVA). Differences were considered statistically significant at $P<0.05$.

Results

Table (1) show the harmful effect of glycerol on MDA and PC as well as the depletion of antioxidant GSH, SOD, and CAT, it seems that pycnogenol can attenuate these effects, the results obtained in table (2) showed reduction in plasma protein, plasma Sodium content in glycerol treated group in concomitant with increase in their Potassium, urea nitrogen, urea, and creatinine, pycnogenol can overcome these effect significantly.

Table(1): Lipid peroxidation product and some oxidative stress markers in control and different treated groups.

Parameter	MDA (nM/mg wt kidney) $\bar{X}$ ± SE	PC (µM/mg wt kidney) $\bar{X}$ ± SE	GSH (mg/g wt kidney) $\bar{X}$ ± SE	SOD (U/g wt kidney) $\bar{X}$ ± SE	CAT (µM H_2O_2/Sec/g wt kidney) $\bar{X}$ ± SE
Control	142 ± 0.8	4.1 ± 0.2	0.31 ± 0.01	128.2 ± 1.3	42.3 ± 0.2
Pycnogenol	140 ± 0.8	3.9 ± 0.13	0.33 ± 0.02	130 ± 1.5	43.5 ± 0.4
Glycerol	425*** ± 0.81	8.1*** ± 0.2	0.09*** ±0.02	95.02*** ± 1.5	13.01*** ± 0.1
Pycnogenol + glycerol	153* ± 4.4	4.9** ± 0.16	0.22** ± 0.01	117.11* ± 2.1	37.1** ± 0.2

$\bar{X}$ = Mean value
P-value = probability
S.E. = Standard error

non significant difference at P>0.05
* = significant at P<0.05
** = significant at P<0.01
*** = significant at P<0.001

Table(2): Plasma protein, some minerals, urea nitrogen, urea, creatinine content and ratio between plasma urea nitrogen content to plasma creatinine content in control and different treated groups.

Parameter	Plasma total proteins content (g/dl) $\bar{X}$ ± SE	Plasma Potasium content (mEq/L) $\bar{X}$ ± SE	Plasma Sodium content (mEq/L) $\bar{X}$ ± SE	Plasma urea nitrogen content (mg/dl) $\bar{X}$ ± SE	Plasma urea content (mg/dl) $\bar{X}$ ± SE	Plasma creatinine content (mg/dl) $\bar{X}$ ± SE	Ratio between plasma urea nitrogen content to plasma creatinine content
Control	5.48 ± 0.37	4.4± 0.1	135.1± 0.2	7.2 ± 0.19	15 ± 0.5	0.51 ± 0.013	8 : 1
Pycnogenol	5.51 ± 0.4	4.45± 0.1	135.3± 0.25	7.4 ± 0.4	15.6 ± 0.1	0.54 ± 0.011	8 : 1
Glycerol	2.44*** ± 0.1	6.0*** ±0.3	124*** ±0.4	37.06*** ± 0.4	75*** ± 0.7	2*** ± 0.06	36 : 1
Pycnogenol + glycerol	4.71** ± 0.5	5.1** ±0.1	130**±0.2	16.4*** ± 1.2	51.3*** ± 0.8	0.9*** ± 0.01	18 : 1

$\bar{X}$ = Mean value
P-value = probability
S.E. = Standard error

non significant difference at P>0.05
* = significant at P<0.05
** = significant at P<0.01
*** = significant at P<0.001

Discussion

Oxidative stress is a term denoting an imbalance between the production of oxidants and the respective defence system of an organism. Glycerol is a well known material for the induction of acute renal failure *in vivo*. It promotes free radicals formation, lipid peroxidation and renal dysfunction, (Zager,1996). The present results showed that, injection of glycerol to male mice caused significant increases ($P<0.001$) in MDA and PC levels compared to control or pycnogenol-injected groups. Similar changes were already observed in different experimental situations designed by Salter and Mullins (1998); and Chander *et al.* (2003). Obtainted increase in MDA in glycerol treated group may be attributed to endogenous toxic oxygen derivatives, such as $OH^{\cdot}$, , Fe^{++} and RNS, a view which is in accordance with Hogg *et al.* (1994). Obtained increase in lipid peroxidation in glycerol treated group may be occur either by release of free ferrous iron , resulting in generation of hydroxyl radicals via Fenton reaction, or by redox cycling of ferric myoglobin to lipid peroxidation, inducing ferryl myoglobin , an explanation which agree with Salter and Mullins (1998). Administration of pycnogenol 30 min. prior to glycerol adminstration led to a significant decrease in MDA as well as PC content relative to that in glycerol treated group. These results are in agreement with that obtained by Giovannini *et al.* (2001), who demonstrated that, pycnogenol administration significantly decreased LPO in kidney of rats. Obtained amelioration in this group may be explained by the ability of pycnogenol to scavenge free radicals and/or inhibit their formation, by direct scavenging of the intiating radicals, especially hydroxyl and superoxide free radicals and regulate excess nitric oxide by quenching the NO radical and inhibiting both NOS mRNA expression and NOS activity as reported by Packer *et al.* (1999). The protective effect of pycnogenol against LPO and as a factor influencing membrane fluidity, may also be related to its ability to scavenge the peroxyl radical ($LOO^{\cdot}$), as reported by Rodrigo *et al.* (2002) a. The decline oxidative stress caused in acute renal failure leading to myoglobinurea by antioxidant pretreatment may be resulted by reduced morphological changes in renal cells as mentioned by Chander *et al.* (2003). GSH content was significantly diminished in glycerol treated mice versus control, this finding is in agreement with those obtained by Singh *et al.* (2004). We can suppose that, the reduction in GSH after glycerol injection might result from the utilization of –SH groups to scavenge free radicals formed as reported by Rahman *et al.* (2005). Administration of pycnogenol was found to improve significantly GSH content. This improvement may be attributed to the ability of pycnogenol to stimulate the synthesis of GSH and strengthen the antioxidant mechanism, an explantation which agree with Dvorakova *et al.* (2006). Activities of SOD and CAT were significant reducded ($P<0.001$) in glycerol treated group. These results are in agreement with those obtained by Pigeolet *et al.* (1990). This depletion may be attributed presumably to the vulnerability of their active centers to free radicals. Administration of pycnogenol 30 min. prior to glycerol injection was found to preserve SOD and CAT activities around the normal values, a result which is in agreement with Bayeta *et al.* (2000) who concluded that, pycnogenol can stimulate several antioxidant enzymes such as SOD and CAT, these amelioration may be due to role of pycnogenol to stimulate cells to produce more antioxidative substances as reported by Yang *et al.* (2008). In the current experiment, glycerol administration to mice results in decreases of plasma proteins. This result is in agreement with those obtained by Packer *et al.* (1999). These changes may related to the effect of ROS promoted by glycerol which cause oxidative damage to proteins. The most straightforward explanation for the depletion of plasma proteins is alteration protein mobility or predispose proteins to endogenous degradation by proteolytic systems, as glycerol administration induce damage in the skeletal muscles and resulting in liberation of all of its componants into circulation and formating free radicals

including NO and either superoxide or hydrogen peroxide which lead to peroxy-nitrite formation and inturn, peroxynitrite reaction with proteins which known to produce several biochemical modifications (i.e., amino acid nitrosylation, sulfhydryl oxidation) as reported by Pryor *et al.* (1995). Membrane fluidity is influenced by proteins present in membranes Karbownik *et al.* (2000). As mentioned by Zager (1996), glycerol seems to damage mitochondrial thiol proteins, it is possible that, the protective effect against the decreased membrane fluidity due to glycerol may relate partially to pycnogenol's prevention of protein damage as reported by Packer *et al.* (1999). pre-treatment of pycnogenol protects against the damage and depletion in plasma protein induced by several oxidative agents, as it can detoxify $OH^{\cdot}$, H_2O_2, NO, $ONOO^{-}$, singlet oxygen and to some degree $LOO^{\cdot}$ (Rodrigo and Rivera, 2002). The present results showed significant changes in plasma electrolytes contents in glycerol treated-mice including decrease in plasma Sodium level and increase in plasma Potasium level as compared to control or pycnogenol-pretreated mice. These finding are in agreement with those obtained by Zager (1996). These changes may be due to mislocalization of ionic pumps, relocation of sodium-potasium ATPase to apical membrane, and cell necrosis, a view which in accordance with Molitoris *et al.* (1992). The estimated decrease in plasma Sodium level may be also due to either plasma membrane disruption or reduced cellular energy; ATP production caused by rhabdomyolosis induced by glycerol administration, decreased in rate of Sodium absorption (hyponatremia) and increased Potasium secretion, an explanation which in accordance with that of Lieberthal *et al.* (1998). The increased plasma Potassium level may be due to plasma membrane disruption and renal tubular cells damage induced by glycerol action which decreases the activity of Sodium-Potassium ATPase and result in decline of Sodium-Potassium pump leading to inability of Potassium exchange, as reported by Araya *et al.* (2001). Administration of pycnogenol was found to preserve plasma electrolytes level around the normal values, as reported by Packer *et al.* (1999). This effect may be due to its ability to prevent Sodium-Potassium ATPase depletion, and keep normal exchange of electrolytes preventing the accumulation of intracellular Calcium and Sodium and ameliorate the mislocalization of ionic pumps, an explanation which in accordance with Rodrigo *et al.* (2002) b. Administration of glycerol significantly increased plasma urea nitrogen, plasma urea and plasma creatinine. These results run parallel with previous reports of Zager (1996) and Singh *et al.* (2004). These alteration may contributed to the retention of nitrogenous substances, by glycerol administeration, causing hyperuremia which result from damaging renal cells leading to accumulation of nitrogen waste. Renal cells failure in filtration and excretion of creatinine in urine may led to retention and increase plasma creatinine level, a view which in accordance with Rodrigo *et al.* (2002) a. Pretreatment of pycnogenol may help to protects against elevation of plasma urea nitrogen, plasma urea and plasma creatinine caused by glycerol, these results probably from several properties of proanthocyanidin found in pycnogenol especially its potent antioxidant capacity which contribute to enhancement of the renal antioxidant defence system, since the kidney is a highly perfused organ as reported by Orellana *et al.* (2002). Pycnogenol containing Proanthocyanidin significantly improve kidney function of mice by elimination of excess fluids and prevent the accumulation of nitrogenous waste products which result in attenuation of the elevation in plasma urea nitrogen, plasma urea and plasma creatinine and improving the renal dysfunction caused by glycerol, a result which in agreement with Avramovic *et al.* (1999). Obtained kidney failure defined as increase the ratio between plasma urea nitrogen content to plasma creatinine content (36:1) as mentioned by Feinfeld *et al.* (2002). It seems that, pycnogenol have relatively protect kidney failure where the ratio between plasma urea nitrogen content to plasma creatinine content reach only 18:1 , a result which confirm the role of pycnogenol in this protection, and may be as a result of normalization of kidney functions. In conclusion it seems that,

glycerol induced acute renal failure may be attenuated to some extent by pycnogenol administration with its antioxidant properties.

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الدور الدفاعى للبكنوجينول ضد الفشل الكلوى الحاد المحدث بواسطة الجليسرول فى الفئران

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يعتبر الجليسرول مصدر داخلي لإطلاق أصناف عديدة من الأكسجين النشط والتي تسبب شد تأكسدي لتراكيب خلايا الكلى.

البكنوجينول عبارة عن مادة طبيعية مستخلصة من قلب شجره بفرنسا تسمى Coastal pine و يعمل كمضاد للأكسدة وقانص للشوارد الحرة. وقد صممت هذه الدراسة لفحص التأثير الوقائى للبكنوجينول ضد الأضرار الناجمة عن الضغط التأكسدى المحدث باستخدام الجليسرول على الفئران، حيث قسم20 فأر عشوائيا تتراوح اوزانها بين30-35جم إلى 4 مجموعات متساوية كالتالى:

أ- مجموعة طبيعية ضابطة.

ب- مجموعة معاملة بالبكنوجينول عن طرق الفم كجرعة واحده قدرها 400 ملجم/كجم من وزن الجسم.

ج- مجموعة حقنت فى العضل بالجليسرول كجرعة واحده قدرها 8ملجم/كجم من وزن الجسم.

د- مجموعة معاملة بالبكنوجينول عن طريق الفم كجرعة واحده قدرها (400 مج/كجم من وزن الجسم) قبل الحقن بالجليسرول بنصف ساعة في العضل كجرعة واحده قدرها (8مجم/كجم من وزن الجسم).

<u>ويمكن تلخيص النتائج كالأتي:-</u>

في مجموعه الفئران المعاملة فقط بالبكنوجينول أظهرت النتائج أن البكنوجينول نشط جهاز الجسم المضاد للإجهاد التأكسدي حيث سجلت الدراسة زيادة غير إحصائية للجلوتاثيون وكذلك نشاط الأنزيمات المضادة للأكسدة مثل , SOD, CATمحتوي البروتين الكلي في البلازما و مستوى الصوديوم فى البلازما. بينما سجلت نقص ذو دلالة إحصائية في المحتوى الكلوي من (MDA) وكذلك (PC) ,مستوى البوتاسيوم فى البلازما وكذلك البلازما يوريا نيتروجين و اليوريا والكرياتينين مما يدل علي أن البكنوجينول مادة غير سامة.في مجموعة الفئران المعاملة بالجليسرول في العضل كجرعة واحده قدرها 8مج/كجم من وزن الجسم فقد لوحظ الأتي:-

زيادة محتوى (MDA) وكذلك (PC) زيادة ذات دلالة إحصائية، بينما انخفض مستوى (GSH) انخفاضا ذو دلالة إحصائية في مطحون نسيج الكلية. بالإضافة إلى انخفاض نشاط الإنزيمات المضادة للأكسدة مثل (SOD) ، (CAT) انخفاضا ذو دلالة إحصائية. في حين أن مستوي البوتاسيوم في البلازما ارتفع ارتفاعا ملموسا، كما انخفض كل من مستوى الصوديوم ومحتوي البروتين الكلي في البلازما انخفاضا واضحا. بينما زاد محتوى البلازما يوريا نيتروجين وكذلك اليوريا والكرياتينين فى البلازما. بالمعاملة بالبكنوجينول عن طريق الفم قبل الحقن بالجليسرول بنصف ساعة ، فقد لوحظ تحسنا ذو دلالة إحصائية في معظم المعايير المقيسة.